

STRUCTURE OF SPORES IN RELATION TO FERN PHYLOGENY¹

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Only in the past quarter of a century have the spores of ferns been used extensively in elucidating systematic relationships. Surprisingly, F. O. Bower (1923–28) made but little use of spores. In his volume I, "Criteria of Comparison," there was no chapter on spores *per se*, and he devoted only a few pages to the subject (pp. 258–272). The trend toward utilizing spore structure was illustrated by Copeland (1947) who used them in characterizing the taxa in *Genera Filicum*. Since then there has been a veritable explosion in the interest in fern spores. The advent of more critical optical equipment, especially the scanning electron microscope, has provided a strong boost to palynological studies in general, especially at the level of species, varieties, and hybrids. For the investigation of spores taken from dried plants on herbarium sheets, the technique of using gummed tape, as described by Martin and Drew (1969), has proved to be especially valuable.

Fern spores have actually been objects of study for well over a century, but many of the earlier papers are overlooked or ignored. Of course some represent only minor contributions, but one of them, the important article by E. Hannig (1911), pioneered the research on the development of the perispore and on the systematic significance of this, somewhat controversial, structure. (Alston's later [1956] application of data from the perispore to classification was long presaged by Hannig.) Among other notable early workers were Fischer (1892), Fischer von Waldheim (1864), and Tchistiakoff (1874), each of whom gave contributions to the knowledge of the morphology and development of fern spores.

Studies of spores of ferns of given localities have been popular for many years. C. B. Weaver in 1895 made one of the earliest of such investigations, "A Comparative Study of the Spores of North American Ferns," in which he illustrated 59 species. In 1935, McVaugh made a similar study of ferns of northeastern United States. Reed's 1953 book on the species of Maryland and Delaware is notable for illustrating not only the plants but their spores as well. More recently, Oliver (1968) described the spores of ferns of Indiana, and Maloney (1961a, 1961b) those of Minnesota.

In other parts of the world we have the works, for example, of Knox (1951) involving British ferns, Sladkov (1959a, 1959b, 1959c, 1961, 1968) on Russian ferns, and Sorsa (1964) on Scandinavian ferns. Especially valuable for the general systematist are the investigations of Selling in Hawaii (1944, 1946), Tschudy and Tschudy in Venezuela (1965), and in particular Harris's book

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(1955) on New Zealand fern spores. Tardieu-Blot's reports (1964, 1965, 1966) on Madagascar are notable for the excellence of the illustrations.

With all of this information now available for geographical areas, there is little surprise that fern systematists have been encouraged to utilize the comparative aspects of spores in arriving at conclusions. Further stimulation to the palynological investigation of ferns by systematists has been occasioned by the appearance of a number of general works such as that by Brown (1960*a, b*) on techniques of preparation, Foster (1956) on microscopy, Kremp (1965) on nomenclature, and especially the book by Erdtman (1957) on pollen and spore morphology of gymnosperms, pteridophytes, and bryophytes. As a result of the interest engendered in spore structure in relation to fern relationships, we have witnessed numerous reports on specific taxonomic groups.³ For example, the genus of woodferns, *Dryopteris*, has been the focus of special attention because of its unusually interesting cytogenetic and evolutionary aspects. Of the students of spore structure in special groups, Nayar and his co-workers (see bibliography) have been exemplary, and they have contributed numerous substantial reports on various critical genera and species-groups.

The present paper is concerned with painting in a broad picture of the structure of spores in relation to fern phylogeny. I wish to examine trends in various characters as well as their relationship to broad phylogenetic questions. The emphasis is upon homospores rather than the highly specialized heterospores, and the terminology used here is mainly traditional. For a fairly conservative use of terminology the reader is referred to Harris (1955: 12-25). Fern spores are usually either tetrahedral or bilateral in over-all form, the laesurae triradiate or linear respectively. The wall layers are produced by elaboration of the exine or exospore, although there may also be a distinguishable outer layer in ferns, somewhat different in nature from the exine, which is referred to as perine or perispore (illustrated best in the dryopteroid and asplenioid ferns). The protoplast contains a single nucleus until the time of germination, and there are proplastids and sometimes chloroplasts present as organelles. Storage materials are visible, usually in the form of droplets. The spores are mostly transparent as seen under the compound light microscope, so it is possible to make out the major structures fairly well. Optical interference, however, often makes it difficult to assess precisely the structure of the surface, and for this reason critical observations on the surface configuration are made best with the scanning electron microscope.

³To illustrate the nature and range of recent work, the following may be noted: Botrychioideae: *Botrychium*, Haeggstrom & Niemi (1964-65). Ophioglossoidae: *Ophioglossum*: Nakamura and Shibasaki (1959); Sladkov (1959*a*). Osmundaceae: Bobrov (1966). Polypodioideae: Kawasaki (1968); N. Pal and S. Pal (1964); S. Pal and N. Pal (1970); *Polypodium*, Evans (1968). Schizaeoideae: Bolkhovitina (1959, 1962); Selling, *Schizaea* (1944). Anemioideae: *Anemia*, Mickel (1962). Adiantoidae: *Cheilanthes*, Knobloch (1966, 1969); *Pteris*, Sladkov (1957, 1959*b*, 1961). Dryopteridoideae: "Davalliaceae," Braggio (1966); *Dryopteris*, Britton (1968, 1972*a*, 1972*b*); Crane (1955, 1960); Kanamori (1969); Mitui (1972*a*, 1972*b*); Reed (1954); *Polystichum*, Daigabo (1967); *Bolbitis*, Hennipman (1970); *Cystopteris*, Blasdell (1963); Hagenah (1961). Asplenioidae: Bir (1966-67*a*); Wagner (1952, 1966). Azollaceae: Fulvio (1961).

TABLE 1. Evolutionary trends in spore structure which seem probable on the basis of present evidence.

Character	Primitive	Derivative
a. Number	256 or more	128, 64, 32, 16, 4
b. Diameter	25–70 microns	70 or more microns
c. Division type	Meiospore	Mitospore
d. Shape	Tetrahedral	Globose
	Tetrahedral	Bilateral
e. Differentiation	Homospores	Heterospores
f. Variation	Narrow	Wide
g. Exine thickness	1 micron	2 or more microns
h. Exine sculpture	Finely sculptured	Coarsely sculptured
	Finely sculptured	Smooth
	Non-ridged	Parallel-ridged
	Unisculptate	Bisculptate
i. Equator elaboration	Weak	Pronounced
j. Laesura	Flush or nearly so	Raised or ridged
k. Perine	Absent	Present
	Corrugated	Smooth
	Cristate	Spinulate
	Cristate	Tuberculate
l. Color <i>en masse</i>	Tan to yellow	Black
m. Organelles	Proplastids	Chloroplasts
n. Amount of food	Moderate	Abundant
o. Resting stage	Present	Absent
p. Survival	Months to years	Weeks to days
q. Germination time	1 week or more	1 day or less
r. Germination medium	Mineral only	Organic
s. Germination pattern	Linear	Triradiate

Any determination of what constitutes a primitive state in the structure of fern spores must be based upon homologies. If, for example, the “perispore” of one taxon has one origin, and the “perispore” of another has another, any phylogenetic conclusions about the perispores of these plants are likely to be erroneous; we cannot say that one is derived from the other, or that one is evidence of affinity to the other. The fact is that fern spores are extremely simple structures with relatively few characters; parallel evolution has probably occurred repeatedly (see below), as well as convergent evolution. The fossil record of ferns is relatively poor. What we know of it suggests that the tetrahedral type of spore is the most primitive (this type was dominant in the Devonian and the Carboniferous). If we compare spore structure in classes of homosporous plants outside of the ferns, we find that once again the tetrahedral spore is the most prevalent. Again, comparison within the ferns shows the tetrahedral condition to be most widespread. Out of 25 taxa at the level of subfamily, the bilateral spore is dominant in 6, occurs approximately equally with the tetrahedral in 2, and is in the minority or is entirely absent in 17.

Using such bases for judgment as given above, the ancestral fern spore may be described tentatively as follows: Tetrahedral, 25–60 microns in diameter, produced by meiosis, 256 or more per sporangium, undifferentiated into heterospores and showing but little inter-spore variation, the exospore ca. 1 micron

thick, minutely sculptured or smooth, with but a single pattern and the equator a simple angle, perispore absent. As seen *en masse* the spores appear yellow to tan and contain proplastids (not chloroplasts). Metabolites are visible within as droplets or small bodies, and the spore is capable of remaining viable to periods of months to years under natural conditions. Germination requires several weeks and is either amorphous at first or unipolar.

Such a description as that above should not be taken in any way as a final declaration of what the original fern spore was like. We must recognize other alternatives, at least as real *possibilities*. Such evolutionary phenomena as reversals and parallelisms have very probably occurred repeatedly and may perhaps have altered the real trends sufficiently as to obscure them. New evidence may change the whole pattern.

Table 1 summarizes a number of evolutionary trends in spore structure which, at present, seem probable on the basis of our evidence. At this point it is hard to imagine what adaptive value bilateral spores may have over tetrahedral, or indeed perispore *vs.* its absence, or most of the other trends. In this regard, the subject of possible adaptive "syndromes" will be discussed below.

Bower (1923: 264) dealt with the trend in spore number per sporangium in considerable detail. When we discover only 32 or 16 spores per sporangium, it may be the result of two processes—the assumption of the apogamous life cycle (discussed later) or an actual diminution in spore number as a result of reduction in number of spore mother cells and the number of archesporial divisions leading up to meiosis. The number 64 is certainly the most widespread in ferns; but such primitive assemblages as Ophioglossaceae, Marattiaceae, and Osmundaceae have much higher numbers, suggesting that reduction to 64 had adaptive significance in evolution. When there are only 4 spores per sporangium, this is associated with megasporangium.

Although no statistical analysis has been made that brings together all the published data, the many reports on spore size in the literature (*e.g.* Harris, 1955; Selling, 1946; Tschudy & Tschudy, 1965) strongly suggest that spore dimensions over 70 microns are derivative. "Standard" fern spores run between 25 and 60 microns. Several factors may be responsible for increase in spore size, of which two are considered below. Typical ferns produce their spores by meiosis in spore mother cells with $2n$ or sporophytic number. However, there are several specializations related to apogamy, in which there are modifications of the cytokinetic origin. In the most common apogamous condition (meiotic apogamy), the sporophyte is cytogenetically a "hybrid," with failure of chromosome pairing at meiosis. Restitution nucleus formation takes place, however, prior to meiosis, thus doubling the chromosome number and making normal sporogenesis possible. The spores have then the same chromosome number as the sporophyte. As a rule, apogamous spores are more irregular than sexual, as shown in Figure 7 and as discussed by Kanamori (1971). Another form of apogamy, which is much less common, involves mitospores. There is no interjection of a restitution nucleus stage, and sporogenesis is entirely mitotic (Evans, 1969).

As indicated, of the two basic spore shapes, the tetrahedral appears to be



FIGURE 1. Bisculptate spore of *Arthromeris himalayanum* (Polypodioideae). Nepal, Fleming, 1961 (MICH).

the most primitive. The trilete and monolete patterns of the laesurae correspond to these shapes and result presumably from the manner of cytokinesis involved in their morphogenesis. Studies of the origins of different over-all shapes have been carried out by Marengo (1954, 1959, 1962). Usually one condition or the other—tetrahedral or bilateral—is fixed for a given genus, but intermediates may occur, especially in connection with hybridity or apogamy. Nayar (1967) has found various intermediate types in the somewhat controversial genus *Loxogramme*, and Selling (1946) reported that in Hawaiian *Botrychium*, *Ophioglossum*, *Cibotium*, *Pteris*, *Coniogramme*, and *Adiantum*—all basically tetrahedral-spored taxa—bilateral spores were found “often” to “very rarely”; conversely in *Marattia*—basically bilateral-spored—he encountered tetrahedral spores, “rarely.”

No one seems to question that the heterosporous condition is specialized in

pteridophytes. It is difficult to imagine reverse evolution when so many factors are involved—endosporic gametophyte development, food storage, fast germination in wet conditions, as well as the extreme size differences. Heterospory should not be regarded as a mere size difference, but instead a profound alteration of the spore apparatus in which the differentiation of two sexes is complete. Alice Tryon and Vida (1967) speak of “incipient heterospory” in *Platyzoma* which McLean Thomson showed had small spores, ca. 32 per sporangium, and large spores, 16 per sporangium (Bower, 1923). Other deviations from uniformity or constancy in spores are usually related to cytogenetic upsets. Most fern spores are fairly uniform and produce a more or less steep normal curve of size distribution. However, hybrids derived from two or more species and which have not undergone polyploidization tend to have extremely variable spores (Wagner & Chen, 1965; Kanamori, 1969).

The exospore is the most valuable source of data thus far in working out evolutionary relationships at the species and generic level. The architecture of the exospore (exine) is extremely diverse, and Gullvåg (1966) has presented a selective review of the fine structure of pollen grains and spores that is pertinent to its study. Pettitt (1966) has made valuable contributions based upon his investigations of exine structure as revealed by light and electron microscopy. It is evident that very thick-walled spores (*i.e.* layers of 2–6 or more microns thickness) are unusual and probably derivative. Certain genera (*e.g.* *Cyathea*, *Pityrogramma*, *Pteris*) possess individual species with strikingly thick exospores (Nayar & Devi, 1966; Tardieu-Blot, 1965). The most widespread exine sculpturing in ferns seems to be either very finely ornamented or essentially smooth. Massive, coarse sculpturings appear to be specialized.

Three features found in the exospores of certain ferns are almost unquestionably derivative—one involving the entire sculpture pattern, another the spore equator, and another the laesura. Figures 1 and 2 show scanning electron microscope photographs of *Arthromeris* and *Drymoglossum*, genera in the Polypodioidae in which there are actually two patterns, one upon the other. This condition, which I call “bisculptate,” involves in these examples spines and pyramids, respectively, these superimposed on different basic patterns. Figure 3 shows photographs of different SEM views of the spores of a species of *Pityrogramma*, one of a number of genera in which certain species display remarkable elaboration of the equatorial region to produce one or more massive, rounded ridges (so-called “spare tires”) around the circumference. Strongly raised or wall-like laesurae are rare (for an example, see Tschudy & Tschudy, 1965; p. 31, figs. 51–52) in ferns and most likely specialized.

Perispore or perine has been and still remains a somewhat controversial subject. Classically the term has been applied to the type of spore covering found in dryopteroid, blechnoid, and asplenioid ferns—corrugated, sac-like, easily destroyed envelopes (*cf.* Nayar & Devi, 1964*b*; Tardieu-Blot, 1966). The perispore begins its formation after the spore has reached full size and the exospore has completed its development; the first evidence of its formation is a brownish, undulate surface covering. The lines of the crests soon become evident as dull, darker areas (Wagner, 1952). In the fossilized state, the perispore

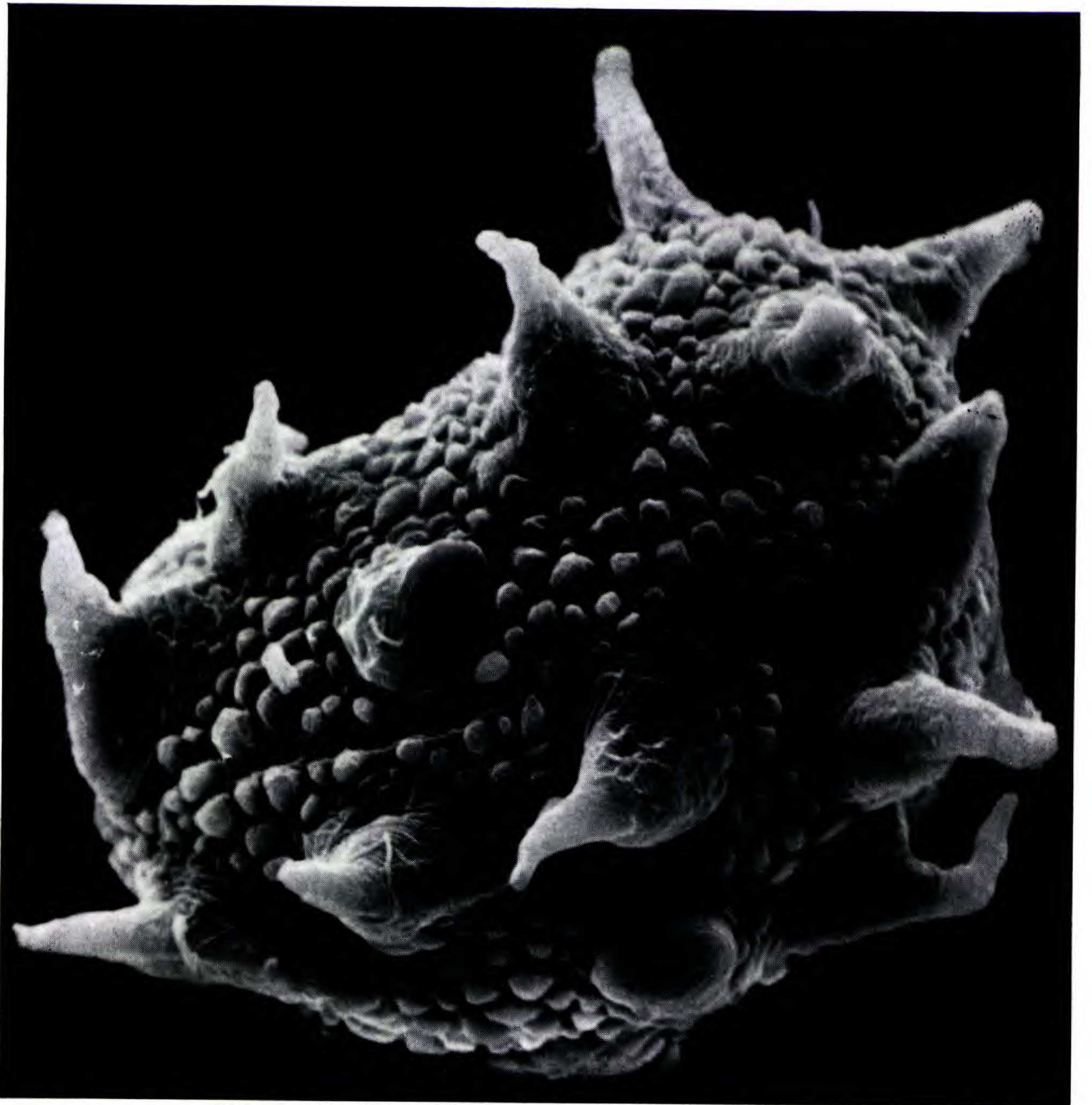


FIGURE 2. Biscalptate spore of *Drymoglossum piloselloides* (Polypodioideae). Philippines, Mendoza, 1953 (MICH).

is absent (Selling, 1946), leaving only the exospore. "Typical" perispores occur in Dryopteridoideae, Blechnoideae, and Asplenoideae, and there are many variations—smooth, tuberculate, cristate, reticulate, spinate. The commonest form, however, looks like the covering of a shrunken prune (*cf.* Tschudy & Tschudy, 1965), and this seems to be the basic type. Some of the best early studies of the perispore were those by Hannig (1911), who was also one of the earliest forerunners to exploit its presence in conjunction with systematics. Pettitt (1966) finds that at least in *Asplenium adiantum-nigrum* "the outer layer . . . or so-called perispore appears, ultrastructurally, to resemble a true exine layer," and he proposes that "there is clearly a need for a full comparative developmental investigation into the formation of these wall layers." On the contrary, the studies of Lugardon (1965) and Hennipman (1970) caused them to recognize a perispore, at least in species of *Blechnum* and *Bolbitis*.

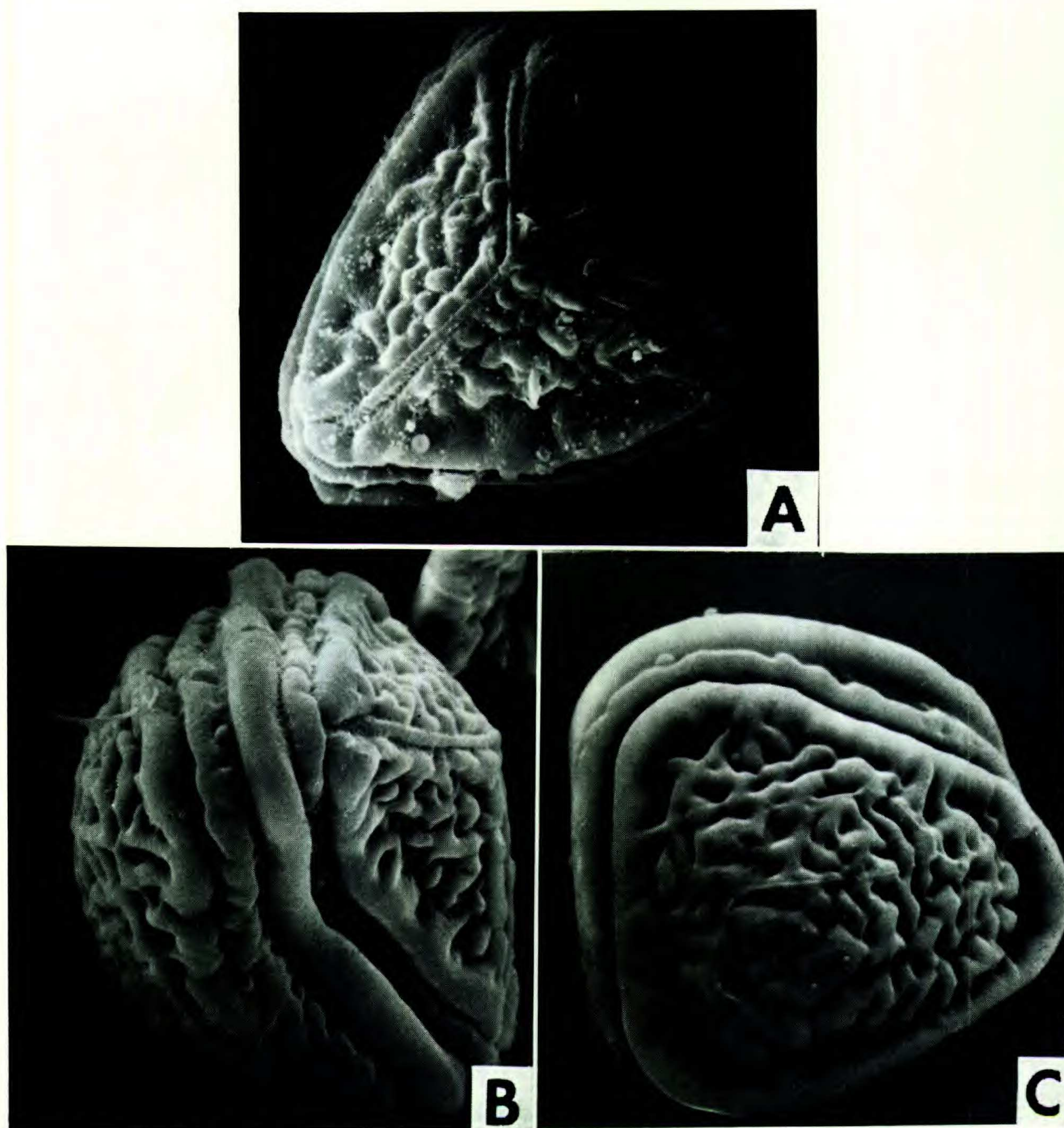


FIGURE 3. Equatorial elaboration shown in three views of spores of *Pityrogramma calomelanos*. Bolivia, *Rusby 130* (MICH).

In any event, the presence of the typical sac-like structure known as the perispore, whatever our ultimate conclusions as to its distinction from the typical spore wall layer or exospore may be, must be considered a specialization. Structures of this sort, so clearly differentiated from the underlying exospore layer, are, except in the indicated subfamilies, extremely rare or absent. The most elaborate perispore structure known to me is that of *Athyrium klotschii*, a species widespread in tropical America. In this species not only are the ridges extremely tall and sharp, but in the depressions, complex vermiform masses of wall material are formed (Fig. 4).

Further research is decidedly needed on the nature of the perispore, and as an introduction to the problems the discussions of Kremp (1965) and Hennipman (1970) are commended.

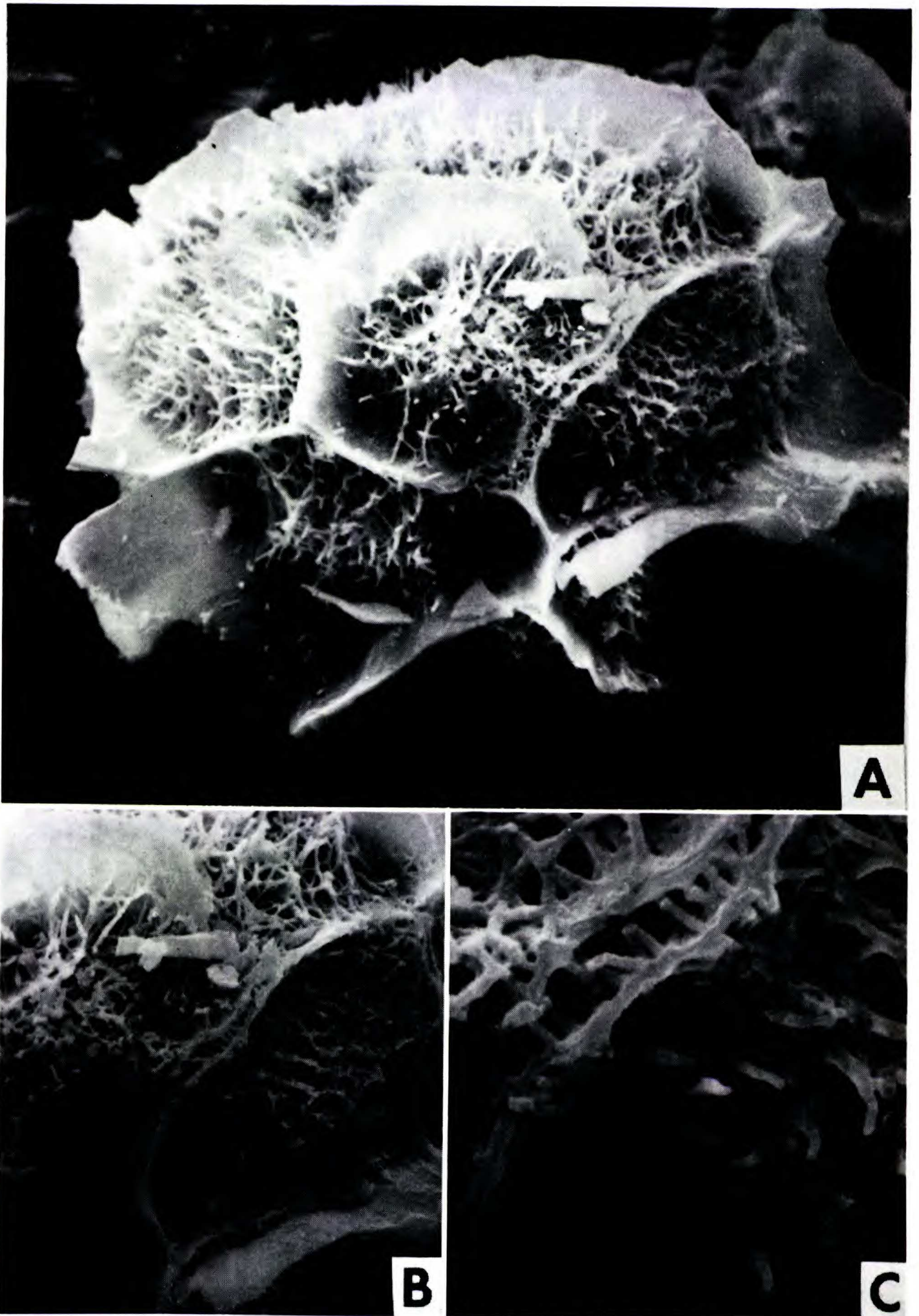


FIGURE 4. Perispore seen at 1500 (A), 4000 (B), and 12,000 (C) times magnification. *Athyrium klotschii*, Bolivia, *Rusby 394* (MICH).

Another characteristic—the metabolite—is also greatly in need of investigation. In general, palynologists are interested in the “shell” and not the organism within; most techniques designed to elucidate the nature of the wall, in fact, tend to destroy the protoplast. Some spores tend to be so “full” of oil drops and other materials that they become optically difficult and the configuration of the wall becomes obscure as seen under the light microscope. The fine structure of primary storage products of the green-spored *Equisetum fluviatile* and the non-green-spored *Blechnum spicant* were examined by Gullvåg (1969), who noted some important differences. Among the comparative data we need on metabolites are the following: (a) chemistry, (b) amount relative to the volume of the protoplast, and (c) form of the inclusions. It might be noted that certain species of both lycopods and of ferns, the spores of which germinate underground, have been found to be especially flammable, igniting easily and producing sheets of fire when scattered in the air and lighted with a burning match, suggesting that they may possess distinctive oils (*cf.* Tulloch, 1965).

The pigmentation of spores may be caused by at least two different factors, namely (a) wall color, or (b) contained chlorophyll. Normally fern spores are yellowish to brown as seen *en masse*. Black spores are relatively rare. Often hybrid perisporial ferns show blackish or deep brown sori and spores, apparently as a consequence of abortion and irregular, sometimes massive, development of the perispore. One of the genera or subgenera of thelypterid ferns—*Amphineuron* Holttum (1971; 45)—is described as having “sporae fere nigrae.” The only member of this group which I have studied myself (*A. gretheri* from Rota, Marianas Islands, Wagner, 1948) had spores which were jet black. Green spores are known in several widely separated taxonomic groups, including Osmundaceae, Hymenophyllaceae, and Grammitidaceae, as discussed by Lloyd and Klekowski (1970). These spores not only have chlorophyll present, but they lack a resting period, germinating immediately in the proper environment. Their walls are thin, and the spores tend toward the globular form. They will be considered below.

Nayar and Kaur (1968) have given us a fine and detailed review of spore germination in homosporous ferns. They conclude the original ferns probably had what is called the “Amorphous type” of germination in which cell divisions occurred in all directions (*e.g.* in Marattiaceae, Matoniaceae, and members of Schizaeoideae). A plate of cells could evolve from an amorphous mass of cells by suppression of divisions in any one of the planes. One of the most distinctive germination patterns involves the “*Hymenophyllum* type,” in which the germling is triangular, arising in a tripolar manner (Stokey, 1940; Atkinson, 1960). Although this could have arisen from the rather rare “Amorphous type,” the possibility should also be entertained that, because of the specialized nature of the filmy-fern life habit, it arose from the “*Osmunda* type” or the “*Cyathea* type,” both of which form “germ filaments,” linear filaments of cells, in germination.

So far we have dealt largely with what might be called “purely morphological trends,” these more or less divorced from their functional or ecological aspects. The structure of spores in relation to fern phylogeny must ultimately

be tied to their adaptive significance, or lack of it. I am of the opinion that when we observe repeated parallelisms and convergences, these are usually of biological significance and tend to improve the survivability of a given line of evolution. What is the adaptive value of few spores per sporangium, of bilateral over-all shape, or of the sac-like perispore? About these we know little or nothing. In regard to other trends, however, there do appear to be fairly convincing correlations that indicate adaptations.

One of these is, of course, heterospory (*cf.* Piérart, 1961). With the gametophytes endosporic and parasitic, and the megaspores provided with much food, heterospory permits rapid fertilization (there being no need to "grow" a gametophyte), and there is abundant food already available for the developing embryo. This condition, so well known in Marsileaceae, Azollaceae, and Salviniaceae, enables species to exist in drought habitats where the ponds dry up. Sporadic rains and formation of pools enable these plants to go through their reproductive cycle. The phenomenon of apogamy also seems to be correlated with dry habitats. In apogamy, the number of spores per sporangium is halved, and through either restitution nucleus formation or direct mitotic division, the chromosome number of the spores is the same as that of the sporophyte (interestingly, triploidy is one of the commonest genome situations in apogameons). It is observed especially in Adiantaceae in such genera as *Pteris*, *Pellaea*, and *Cheilanthes*, and because of the ability to produce a sporophyte by direct proliferation from the gametophyte, it enables the plant to reproduce by spores but without dependence upon free water. All that is required is a humid environment, and water for the sperms to swim in may be lacking. These plants occur most commonly in well drained habitats or dry, often rocky, cliffs.

As noted earlier, large spore size, *i.e.* size that is amplified well beyond the normal for the given species or species-group, is evidently derivative. There do seem to be at least two biological correlations involved in large spores, these unrelated to one another. The first and best-known is the change in spore size associated with multiplication of the basic chromosome number due to doubling one or more times (*e.g.* Bir, 1966-67*b*; Blasdell, 1963; Kanamori, 1971; Wagner, 1970). Where normal spores are produced by these polyploids, their cytogenetic constitution appears to be either $A^1A^1A^2A^2$ (*i.e.* autopolyploids in which different pairing factors enable normal meiosis) or $A^1A^1B^2B^2$ (*i.e.* allopolyploids with different pairing factors). Most taxa at the subfamily-family level are known to have polyploids. The polyploids in *Ophioglossum* are noted for their huge numbers—up to $2n$ over 1200! Only, no polyploids have yet been reported in Osmundaceae. The other correlation involving large spores is the one detected by Sherwin Carlquist in his studies of loss of dispersibility in the Hawaiian flora. Carlquist (1966) found that unusually large spores occurred in certain taxa (Schizaeoideae, Hymenophylloideae, Adiantoideae, Blechnoideae) in members which occurred in the rainy, high forest of the islands of the Hawaiian archipelago. According to his theory, the production of unusually large spores counteracts the wide scattering of spores to unsuitable low, dry areas (or out to the high seas). The spores thus tend to fall near by, on the same or adjacent high rain-forest ridges.

The possible biological significance of chlorophyllous spores has been analyzed by Lloyd and Klekowski (1970). These spores, which are found in Osmundaceae, Grammitidoideae, Hymenophylloideae, as well as *Onoclea* and *Matteuccia* in the Dryopteridoideae, are characterized by thin walls, a more or less globose outline, short viability, and quick germination. Rather than proplastids they contain chloroplasts. I am impressed by two correlations—one in space, the other in time. Green spores occur in the rain forest (grammitids, filmy-ferns) in the tropics, where they are produced, presumably, year-round. Green spores also occur in the temperate zones (osmundas, onocleoids), but here they are produced in the spring only—the period of maximum rain. In both situations, the large amount of available moisture is evident. Thus the spores will not be damaged by the environment (as they would in drier places or times), and there is no need for resistance. This type of spore apparatus allows for immediate germination, and there is no resting stage.⁴

A type of spore which has long baffled the experimental morphologist is characterized by its nearly ungerminable behavior in culture. Only recently have techniques been developed to germinate *Botrychium* spores, for example. In the laboratory of Dean P. Whittier (Vanderbilt University, Nashville, Tenn.) great strides are being made toward germinating spores of this type and growing their gametophytes (Whittier, 1972, 1973). Nevertheless, it is clear these spores, which are found in Botrychioideae, Ophioglossoideae, certain Schizaeoideae (and also lycopods), have a distinctive behavior under natural conditions, as well as under culture conditions involving purely mineral media. The walls seem to be very resistant and the contents, as seen under the light microscope, commonly show a profusion of oil droplets (*cf.* Tulloch, 1965). Germination in nature apparently requires fungal symbiosis. Germination-resistant spores are correlated with buried habitats, lacking light, and having suitable fungal components for forming mycorrhizal relationships. By making the necessary evolutionary changes, germination becomes delayed so long as to enable the spore to percolate into the soil, and there establish symbiosis. This explains why so many of the gametophytes of grapeferns, adder's-tongues, and clubmosses are found 1–5 cm below the soil surface. They obviously did not sink to those levels after germinating at the surface.

Any adaptive correlation for bilateral spores is at this point difficult to detect. However, there is one group of bilateral spores which may prove to be adaptive, although this is not yet clear. It involves the type of spore so common in Poly-

⁴ In studies of times of spore release in pteridophytes of the Great Lakes area, which I have been carrying out in collaboration with Royce H. Hill, we find that not only the osmundas (3 spp.) and onocleoids (2 spp.), but the equisetums as well (9 spp.) tend to discharge their spores in April and May (June in northern Michigan). The fact that horsetails and scouring-rushes, which also have chlorophyllous spores, tend to release them at the same time reinforces, we believe, our conclusion of the adaptive significance. Further reinforcement comes from an examination of the spores produced in the driest season of the year (August, September): The latest spores to be produced by pteridophytes of Michigan are those of 4 out of 10 species of *Botrychium* (all members of subgenus *Sceptridium*), and all 11 species of *Lycopodium*. These, of course, represent the taxa which possess mycorrhizal gametophytes which are more or less subterranean, and which are *slowest* to germinate.

podioideae and Dryopteridoideae in those members which may be termed "dry epiphytes." These include such genera as *Polypodium*, *Pyrrosia*, *Davallia*, *Humata*, and *Nephrolepis*, the species of which tend to grow in dry tree-canopy in epiphytic situations in tropical regions, usually in the rain shadow or at middle or low elevations. They may grow also on rock cliffs. As can be shown in a comparison of polypodioid ferns (Nayar & Devi, 1964a) and davallioid ferns (Tardieu-Blot, 1964; Braggio, 1966) there are some remarkable resemblances between the spores, especially the types with shallowly tuberculate or mamillate sculpture. This may constitute a case of convergence, as the relationships of the polypodioid ferns are with families lacking the typical perispore, while the davallioids are evidently derived from dryopteroids with the typical perispore. Thus the davallioids may have lost or greatly reduced this structure, the end-result being a spore strikingly similar to the unrelated, but ecologically similar, polypodioids.

In an interesting statistical analysis, Ito (1972) found that in eastern Asia and northern Oceania there is a correlation between the ratio of monolete:trilete spores (m:t ratio) and geography. The values of m:t increase in accordance with the latitude and altitude in the northern hemisphere, according to his results, the trilete ferns being more abundant in the warmer regions, the monolete in the colder regions. In the Philippine Islands $m:t = 1.27$, but in eastern Himalaya $m:t = 2.25$, and in Japan $m:t = \text{ca. } 2.71$. Following his procedures (*i.e.* eliminating "fern allies," heterosporous ferns, and hybrids), I find in Hawaii (with a total of 128 native ferns) that the ratio $m:t = 1.66$, roughly equal to his ratio from the Bonin Islands and the Ryukyu Islands. However, in the state of Michigan with 63 species, and fairly representative of the north-central region of North America, I find a ratio of 1.74, considerably lower than I had expected. Part of the reason for this result is the unusually rich representation of ophioglossoids in the Great Lakes region. And this also points up the critical importance of taxonomic interpretation in arriving at conclusions of this type. Phytogeographers will recognize immediately the enormous relative increase in representation southward of groups such as cyatheoids, (Cyatheoideae), filmy-ferns (Hymenophylloideae), and dwarf polypodies of the rainforest (Grammitidoideae)—all groups with mainly or exclusively tetrahedral spores. Using Harris's (1955) data, the ratio for New Zealand (152 spp.) is $m:t = 0.97$.

Many fern taxa, especially in the temperate zones, are characterized by extremely variable spores, variable both in their shapes and their sizes. The majority of these spores lack protoplasts and are patently abortive, unable to germinate or reproduce the fern. A few of the spores do, however, contain protoplasts and will form gametophytes (Morzenti, 1962; DeBenedictus [née Morzenti], 1969), and in some instances the percentages of germination are surprisingly high, reaching as much as 36 per cent (Whittier & Wagner, 1971), even though, in practically all examples investigated, the ferns involved are interspecific hybrids. Taxa with the majority of their spores abortive are correlated in occurrence with an association of two species of close relationship which are capable of forming hybrids. Interspecific hybrids have been found

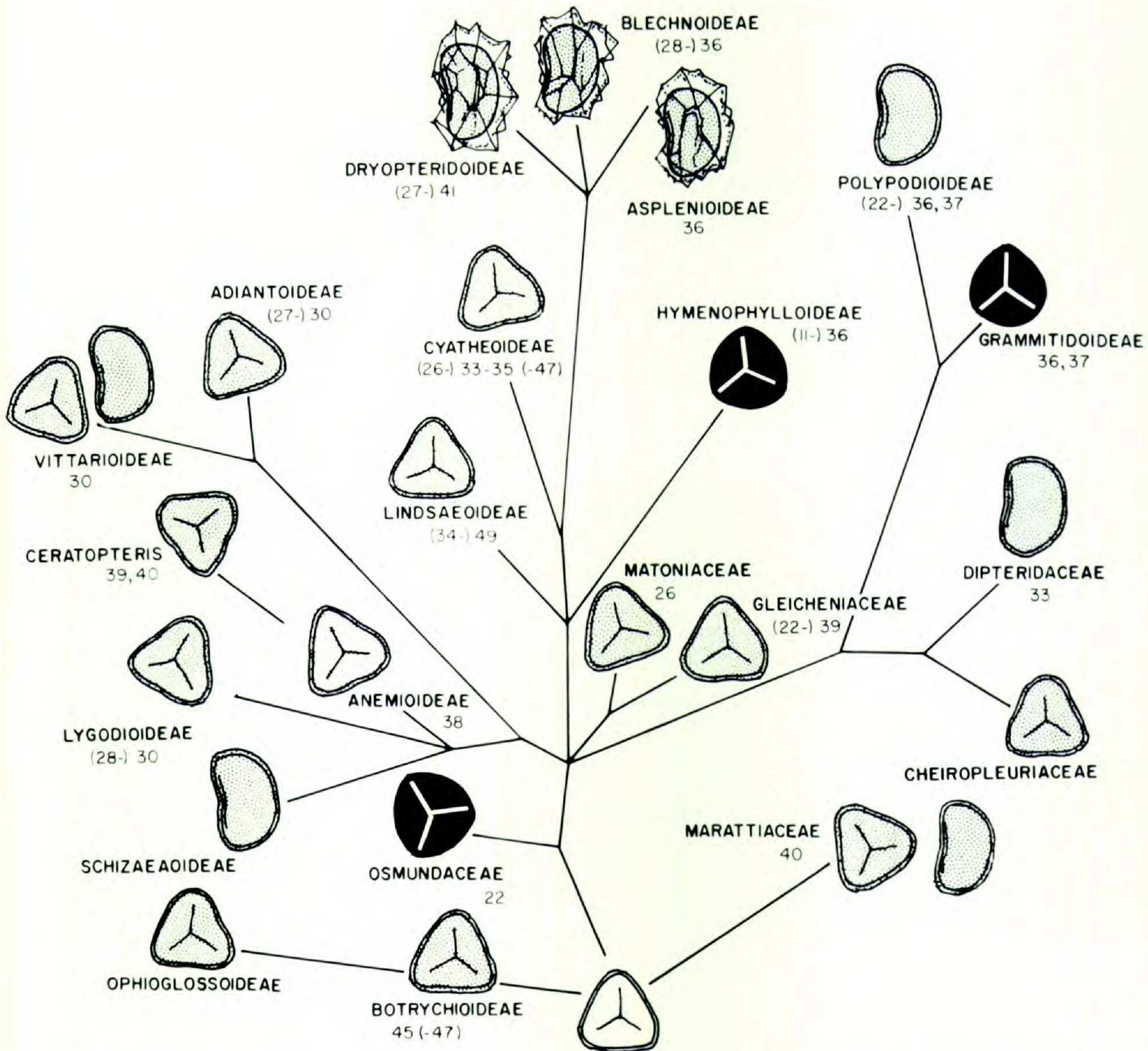


FIGURE 5. Correlation of spore types with phylogenetic groupings of families and subfamilies of ferns. Chlorophyllous spores shown in black; perinous spores with angular covering. Possible basic chromosome numbers shown in parentheses under the taxa.

in practically all subfamilies of ferns, but they seem to be relatively rare in tropical epiphytes. Presumably sterility barriers arise due to accumulated genetic differences between the parents, but they may also be adaptive to the extent that they oppose breakdown of the respective fitnesses of the parental species by the prevention of sloppy combinations of characters.

SPORES AND OVER-ALL PHYLOGENY

That the spores may be useful in solving controversial problems of relationship is unquestioned. In my own research, I have found them especially useful (*e.g.* Wagner, 1952); in the Hawaiian genus *Diellia*, associated by many authors with the lindsaeoid ferns, the spores showed convincingly that the relationships were elsewhere. As in all systematic data, however, it is imperative that the evidence from this one source be correlated with that from other sources. In recent years the study of spore abortion has become a valuable tool in detecting taxa of hybrid origin (Kanamori, 1969, 1971; Morzenti, 1962; Reed, 1954; Wagner,

1954, 1966, 1970; Wagner & Chen, 1965; Wagner & Whitmire, 1957). This use of spores is limited to the level of species-groups closely related enough to make viable hybrids. At the level of genus, family, and higher, spore abortion is of no significance. In the overall phylogeny of ferns we ask questions like, "What are the relationships of the genus *Loxogramme*?" The genus *Cheiropleuria*? These and a number of other genera have been controversial as to their evolutionary position. It is interesting to note that Nayar (1963) investigated spore morphology in *Loxogramme*—a genus regarded by many as more or less intermediate between the bilateral-spored polypodioids and the tetrahedral-spored grammitids—and discovered one species, *L. lanceolata*, in which both spore types exist as well as all transitions between. As regards the genus *Cheiropleuria*, associated with polypodioid ferns by various authors in spite of its many deviant characters, the spore is so different that close relationship is very improbable (*cf.* Nayar & Devi, 1964a).

The broad outlines of correlation of some of the major spore characteristics with presumed phylogenetic relationships are now fairly well known, and are illustrated in Figure 5. The diagram shows that in general a given taxon at the level of subfamily has mainly or exclusively one type or the other—tetrahedral or bilateral. The tetrahedral type is the most prevalent. Bilateral spores are scattered in unrelated groups, indicating parallel evolution. Likewise, green spores are scattered and arose, no doubt, by parallel evolution and do not indicate affinity. Some of the groups, such as Gleicheniaceae and Cyattheoideae, show dominantly tetrahedral spores, but there are bilateral-spored members. Others, such as Vittarioideae and Marattiaceae, have fairly equal representation of both types. In Marattiaceae, genera centering around *Angiopteris* have tetrahedral spores, while those around *Marattia* and *Danaea* have bilateral. In Vittarioideae, there is some suggestion that the genera which exist in the wettest forest possess the tetrahedral type of spore and those which occur in dry forest as "dry epiphytes" (see above) have evolved the bilateral spore; this is an hypothesis that can be tested.

SOME PROBLEMS IN THE USE OF SPORES

We have already indicated above some of the problems that are met in the study of spores. Caution must be observed in regard to a variety of spore characters, and there are the ever-present problems of confusing directions of evolution, reversals, parallelisms, and misinterpreting the extent of variability. As pointed out earlier, there are two entirely different factors that may produce large spores—selection for "precinctiveness" in montane rainforests and polyploidy. (In my original studies of Hawaiian fern spores (Wagner, 1961), I thought that the large spores of the higher rainforests resulted from polyploidy; not until I actually studied the chromosomes did I realize that some other factor was operative.)

In regard to the shape of the spores there may be reversals. At least in one case, that of *Thelypteris*, a genus or genus-group all of the relatives of which possess bilateral spores, there is the suggestion of reversal in a small species-

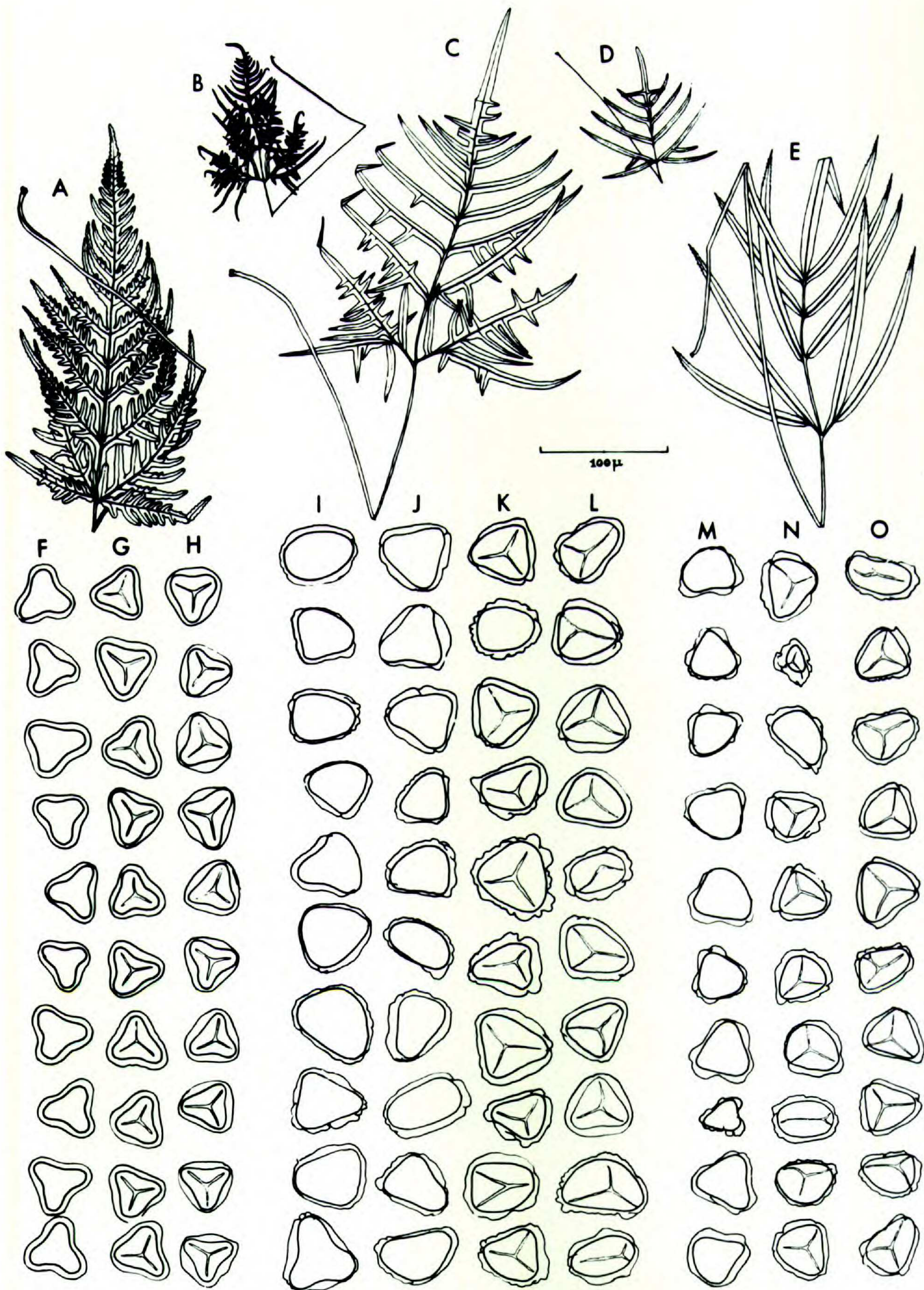


FIGURE 6. Spore tracings (left hand columns drawn without the laesurae) of sexual and apogamous Hawaiian ferns. *Pteris irregularis*—sexual species: A, frond; F–H, spores. *P. cretica*—apogamous species: E, frond; M–O, spores. *P. x hillebrandii*—apogamous hybrid: B, C, D, frond variations; I–L, spores.

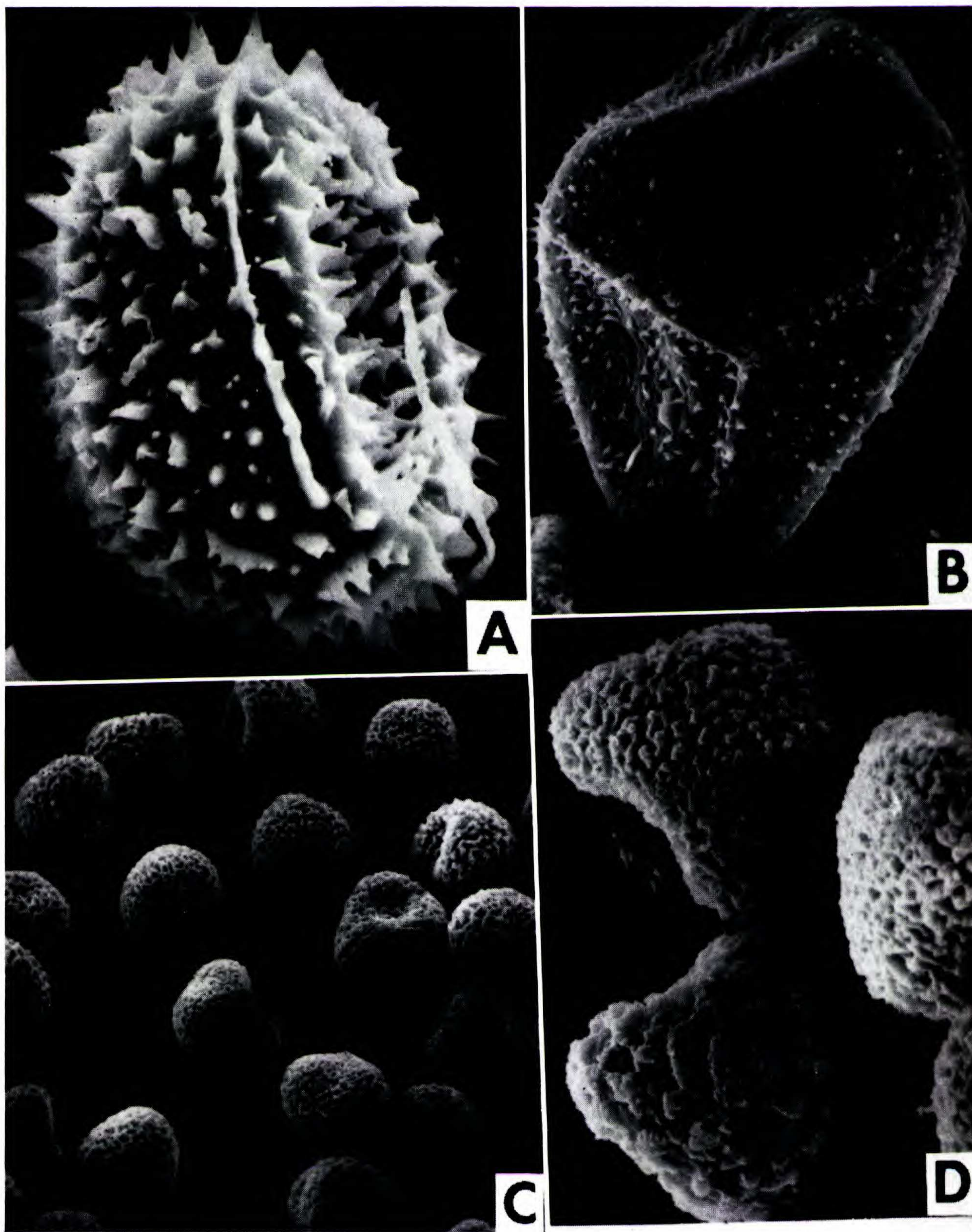


FIGURE 7. Spores.—A. *Thelypteris* sp. India, Chand 4692 (MICH).—B. *Thelypteris* (*Trigonospora*) *ciliata*. Hainan, Lau 5306 (MICH).—C–D. *Botrychium dissectum*. Michigan, Wagner 9101 (MICH).—C. Seen at 600 \times .—D. Seen at 1800 \times .

cluster which Holttum (1971) has recently designated *Trigonospora*. Figure 7 shows a typical thelypteroid spore (A) in comparison with the spore of *Thelypteris* (*Trigonospora*) *ciliata* (B).

The problem of the homologies of the perine or perispore alluded to above has been exacerbated recently by the report of Lloyd (1969) of a “winged

perispore" in *Polypodium chnoodes*. Nayar and Devi (1964a: p. 363, fig. 84) found what is apparently a similar structure in the spores of *Pyrrosia nummularifolia*. Also, in what is presumably a single species, one of the best known fern species in the world, the fragile-fern, *Cystopteris fragilis*, there are evidently two wholly different spore forms, one of which has the typical perispore of dryopteroid ferns ("*C. dickieana*"), the other the characteristic spinate structure of the genus as a whole (cf. Blasdell, 1963; Hagenah, 1961). In *Davallia*, *Humata*, *Nephrolepis*, genera of obviously dryopteroid affinities, the spores resemble those of polypodioid ferns, as discussed earlier. Does this mean that they have lost the perispore? Or is it actually present in vestigial form?

The problem of variability can usually be met when dealing with living ferns, but single fossil spores can conceivably be misleading. To illustrate this point, one need only remove the contents of a single sporangium of certain species of grapeferns, *Botrychium*, and compare the different wall patterns. Figure 7, C and D, shows what we find in the common evergreen grapeferns of the eastern United States (cf. Sladkov, 1968).

In addition to the parallelisms already discussed, one additional one should be noted for its very striking character. This one, discovered by Sladkov (1959b), involved two wholly different genera, *Botrychium* (Ophioglossaceae) and *Cryptogramma* (Adiantaceae). As he shows (1959b: table 1, fig. 2), their spores are remarkably similar in size, shape, form of the laesurae, and sculpture. It would be easy indeed to confuse them.

CONCLUSIONS

In spite of relatively simple structure, spores in ferns may potentially provide a goldmine of systematic evidence, both at the level of species and higher. The variables, as discussed here, include such characters as number per sporangium, size, cytokinetic origin, general shape, heterospory, variability, nature of the exospore including its sculpture, equator, and laesura—the perispore, pigmentation, organelles, metabolites, resting stage, survivability, and germination. We have attempted to trace here some of the trends in spore morphology in relation to fern phylogeny. For some of the observed trends, we have also examined possible adaptive values. With new information, it is possible that some of the trends dealt with here will be found to be mistaken, and that, in fact, the direction of change has been the reverse of that postulated. There may be parallelisms and (or) convergences. There may even be evolutionary reversals. Accordingly we must observe due caution in utilizing spores for making phylogenetic conclusions.

Assuming that in general our deductions have been correct, the broad outlines of spore structure in relation to fern phylogeny are now drawn. There is a good overall correlation of spore type with the family-subfamily levels of classification. Among the different characters of fern spores, over two dozen probable trends can be distinguished. Not only should we be concerned with testing these with all the evidence that we can marshal, but we should also ask the basic question of what are the reasons that these changes have come about biologically.

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